BLOSSOM *and* TWIG BLIGHT *of Low-bush Blueberries*

(Botrytis Cinerea)

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SUMMARY

TWIG and blossom blight is sometimes a very destructive disease of blueberries in Maine. The disease is caused by *Botrytis cinerea* which infects blossoms, small fruits, twig tips and stems of low-bush blueberries under conditions of high humidity and low or moderate temperature.

Laboratory experiments show that the temperature growth range of the causal organism is between 5 and 32° C., with maximum growth at 23° C. The optimum temperature range of conidiospore germination is from 8 to 29° C.

It was found that the conidiospores are able to overwinter in a dry atmosphere and that the mycelium, after overwintering at natural outside temperatures and humidity, is able to sporulate within 24 hours when conditions are favorable.

After nine days of incubation at high humidity the organism will infect all stages of floral bud development, including the dormant buds. Three to four days of high humidity are necessary for infection of blossoms.

Symptoms of winter twig injury, salt spray injury and frost injury, and the effect of lack of pollination, can be easily mistaken for blossom or twig blight.

A wide variation in susceptibility was found between clones; however, the stems within a clone were found to be almost equally susceptible.

This difference in clonal susceptibility makes it difficult to estimate the prevalence of the disease in the field. One average field was sampled by two methods. A random sampling method, where 100 one-square-foot samples were taken in each of three 3-acre plots, demonstrated a slightly higher per cent of infection than a systematic sampling method, where 100 nine-square-foot samples were taken at regular intervals in three 3-acre plots.

Statistical treatments of data on per cent of disease in replicated nursery clones gave evidence that there was a higher per cent of infection on plants grown under peat mulch than on plants of the same clones grown under sawdust mulch.

Fertilizer treatments also apparently make some difference. More infection was found among plants receiving early fertilization than those fertilized late. Where fertilizer ratios were compared, a higher per cent of infection was found with a more complete ratio.

Forty-one fungicides and 12 antibiotics were tested in laboratory assay against conidiospores. That concentration of ma-

terial which inhibited the germination of 50 per cent of the spores was used as an index of comparison. The elemental sulfur compounds were the least effective; the organic mercury compounds and the dithiocarbamate compounds were the most effective.

A greenhouse fungicidal assay of some of the materials was made in 1952 and 1953. The materials were sprayed on blueberry blossoms and the blossoms were inoculated with a spore suspension. After incubation in a high-humidity chamber, the per cent of disease control was recorded. In general, the dithiocarbamate compounds gave the best control, and the elemental sulfur compounds gave the least. Some organic mercuries, Crag 341, and some copper materials were toxic to blueberry leaves.

In 1952 and 1953, materials which showed sufficient promise in laboratory and greenhouse tests were tested in the field. Since very little disease was present in the field, it was possibly only to obtain data which showed trends of disease control. The results tend to substantiate the laboratory and greenhouse tests.

BULLETIN 529

BLOSSOM AND TWIG BLIGHT OF LOW-BUSH BLUEBERRIES

E. Neil Pelletier and M. T. Hilborn[1]

INTRODUCTION

BLOSSOM and twig blight has perhaps always been present in some degree in Maine low-bush blueberry fields, but it received little attention until recent years when this blight became very important because of weather conditions. The disease is caused by *Botrytis cinerea* Pers. In some years only the blossom blight phase of the disease appears and in other years only the twig blight phase. Sometimes both phases occur simultaneously and are quite widespread in blueberry fields.

This bulletin[2] presents the results of research on three aspects of the disease, (1) seasonal development, (2) the effect of environmental factors, and (3) chemical control. The work was supported financially by Maine Blueberry Tax funds.

The fungus is able to kill blossoms, immature fruit, young tips and stems. The fungus may progress downward through the corolla into the young fruit and thence into the stem. It may also enter injured stems or the dead tips of stems showing determinate growth and progress further along the stem. Leaves may be infected whenever they come into contact with infected blossoms or twigs. A period of high humidity and low or moderate temperature at the same time the plant is most susceptible is optimum for the fungus, whereupon a high per cent of the blossoms and consequently the fruit may be lost.

REVIEW OF LITERATURE

THE first reference to *Botrytis* blueberry blight in Maine was made by Markin (22)[3] who reported that *Botrytis* blossom blight was very common in 1930, appearing during a week of foggy, warm weather in late June. *Botrytis* was seen to sporulate on occasional shrunken berries during the summer. The blight was observed on *Vaccinium*

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[2] Part of the work reported includes work toward an M.S. degree in the Graduate School, University of Maine. Special recognition for assistance in this work is due Dr. Donald Folsom, Prof. Moody Trevett, Mildred R. Covell and Dr. Lincoln Taylor.

[3] Numbers in parentheses refer to Literature Cited, page 25.

canadense Kalm (*myrtilloides* Michx.), V. *Corymbosum* L., and *V. Pennsylvanicum* Lam. (*angustifolium* Ait.). In a Maine Station miscellaneous publication (2) it was noted that no conclusive results were obtained in 1947 in blight control experiments, but that dusting with 20-20-60 copper-arsenic-lime dust (in 1947) seemed to reduce the amount of overwintering twig blight in 1948.

The blight was found by the second author in 1949 to be mistaken for winter injury and several fungi were found associated with the disease. In 1951 blossom and twig blight was one of the principal blueberry diseases in Maine and was causing serious losses. The organism causing blossom blight also caused one type of twig blight, and preliminary tests by the Maine Station indicated that Fermate, applied at the cluster bud stage, would aid in its control.

Demaree and Wilcox (9) in a 1947 study of fungi pathogenic to blueberries in the eastern United States rated *Botrytis* as sometimes a cause of considerable loss, noting that it attacked blossoms, fruits and leaves during prolonged periods of foggy weather. Goheen (14) in 1950 found that *Botrytis* was the primary invader causing an extensive twig blight and associated fruit rot and leaf spot of blueberries in western Washington. Hockey (10) stated in 1950 that moist seasons favor twig blight and fruit rot caused by *Botrytis* on low- and high-bush blueberries in the Canadian Maritime Provinces. Darrow and Demaree (8) indicated that twig blight caused by a species of *Botrytis* was the most important blueberry disease in the Pacific Northwest. The fungus also produced blossom blast, leaf spot and fruit rot in wild blueberry species as well as in cultivated high-bush species.

THE CAUSAL FUNGUS

ACCORDING to Whetzel (36), *Botrytis cinerea* is an imperfect stage of *Botryotinia fuckeliana* (DeBary) Whetzel. A culture isolated from blueberry in 1952 was sent to Dr. F. L. Drayton at the Central Experimental Farm, Ottawa, Canada. In reply, it was stated that "the culture . . . appears to be morphologically the same as *Botrytis* of the *cinerea* type from which we have produced the perfect stage of *Botryotinia fuckeliana,* although we have not established a connection between *Botrytis* of the cinerea type and *Botryotinia* using isolates from blueberries." Dr. W. Lawrence White of the Farlow Herbarium, Harvard University, and Dr. E. E. Honey, Pennsylvania State University, also examined cultures of this organism and both classified it as in the genus *Botryotinia.*

Hawker (15) pointed out that the formation of conidiospores by the organism was favored by a relatively low carbon-nitrogen ratio in

the medium. Of the two common culture media, potato dextrose agar, with a relatively high carbon-nitrogen ratio, and nutrient agar, with a relatively low ratio, only the latter was found to give an abundant supply of spores. Spore production on potato dextrose agar was meager. Therefore, whenever an abundant supply of spores was necessary, nutrient agar was used.

The temperature range of spore germination was determined. Twenty-day-old spores were removed from nutrient agar plate cultures and suspended in a spore stimulant solution composed of 0.2 per cent sucrose and 0.001 per cent sodium citrate. One drop of this suspension was applied to a hanging-drop microscope slide by means of a dropping pipette. The slides containing the spore suspensions were incubated in moist chambers at nine different temperatures ranging from 2 to 36° C. After 24 hours of incubation the spore germination per cent was recorded. At each temperature the tests were repeated until two germination percentages were obtained which were less than five per cent apart. There was no germination at the temperature extremes tested. The optimum germination (75 per cent or more) occurred from 8 to 29° C. The germination percentage was plotted on graph paper against the temperature (Fig. 1). Hawker (15) reported that spores of *Botrytis* germinate best between 20 and 25° C. and that 35° C. is the maximum temperature in the absence of a germination stimulant solution. Moore (26) reported that 60 to 68° F. (16 to 20° C.) is the most favorable temperature range for infection by this fungus.

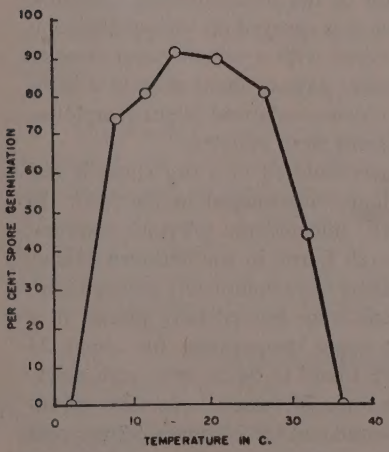

FIGURE 1. Influence of temperature on spore germination of *Botrytis cinerea.*

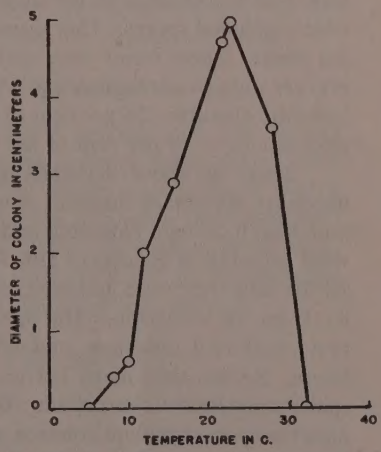

FIGURE 2. Influence of temperature on the mycelial growth of *Botrytis cinerea.*

The temperature growth range of the fungus mycelium was also determined. Potato agar plates were inoculated with the fungus and incubated at nine different temperatures ranging from 2 to 32° C. After 72 hours of incubation the diameter of the colony growth was measured. Repeated trials showed no colony growth below 5° or above 32° C. The maximum diameter growth of five centimeters occurred at 23° C. The colony diameter was plotted on graph paper against the temperature (Fig. 2). As the curve shows, the growth rate declines rapidly with increase in temperature above the optimum. Hawker (15) indicates the optimum growth temperature of *Botrytis* to be 20° and the maximum 30° C. Brooks and Cooley (4) in their study of temperature relations of apple-rot fungi found that the optimum growth temperature for *Botrytis* on corn meal agar was about 25° C.

PATHOGENICITY

TO discover the way in which *Botrytis* is able to cause new infections after the winter season, isolates were made from blueberry twigs, blossoms, and fruits which had overwintered in a dry condition in an unheated outdoor garage at winter temperatures which fell below –18° C.

These overwintered plant materials were surface sterilized and embedded in potato agar plate cultures. After four days of incubation eight isolates of *Botrytis* were made from 17 fungus growths. Conidiospores scraped from some of the overwintered berries were germinated in germination stimulant solution. Sixty per cent of the spores germinated after 24 hours of incubation in a moist chamber. A Waring blender was used to obtain a water suspension of the overwintering materials, which included spores. This suspension was sprayed on several blossoming stems. These stems were then covered with a waxed paper cone to prevent outside contamination. After five days of incubation in a high-humidity chamber, 25 per cent of the blossoms showed blight symptoms; after ten days, 50 per cent of the blossoms were infected.

Since the above materials had overwintered in a dry state, it was necessary to obtain material which had overwintered in the field. In mid-March, stems, blossoms and fruits, infected the previous summer, were gathered at Blueberry Hill Research Farm, in southeastern Maine. At the time they were gathered, the plants were completely covered with ice from an ice storm. The specimens were immediately placed in a pint cardboard container and left at room temperature for about 24 hours. By this time many berries were found to be covered with newly sporulating *Botrytis* mycelium. When some of these spores were germinated in spore stimulant solution and incubated for 24 hours, 93 per cent germination resulted.

These data show that *Botrytis* is able to overwinter in the field as mycelium in infected tissues and in a dry atmosphere as conidiospores. Sporulation occurs in overwintered material in a short time when temperature and humidity are favorable.

The facts that sporulation may occur as soon as temperature and humidity permit and that sporulation occurs more frequently than infection, suggest that the condition of the host is also a highly important factor in infection. To determine at what stage the host is subject to infection and what length of time of high humidity is necessary for infection, three experiments were made.

Infection During Dormant Stage

Four sets of blueberry stems were inoculated with a spore suspension and were incubated in a high-humidity chamber that maintained 100 per cent relative humidity almost constantly. In each set there were three stems at different stages of floral bud development, namely, the dormant stage, the swelling bud stage, and the opening bud stage. The sets of stems were incubated for different lengths of time—Set 1, four days; Set 2, five days; Set 3, six days; and Set 4, seven days. At the end of the incubation periods, Sets 1 and 2 showed no blight symptoms and later developed normally. The swelling and opening bud stages of Set 3 showed mycelial growth, but the dormant bud stage showed none. All stages of development of Set 4 showed mycelial growth. Apparently all stages of floral bud development are subject to infection if humidity is maintained. It would also appear that a period of more than five days of high humidity is necessary for infection of opening buds and a period of more than six days is necessary for infection of dormant buds.

Infection During Flowering

Three stems at different stages of floral development, the swelling bud stage, the opening bud stage, and the opening flower stage, were inoculated with a spore suspension. The plants were covered with a waxed paper cone to prevent contamination and were then incubated in the high-humidity chamber. After nine days of incubation all stems showed some infection, showing that all stages of floral development are subject to infection if high humidity is maintained long enough.

Infection During Full Bloom

Six sets of blueberry stems were inoculated with spore suspensions and incubated in the high-humidity chamber. There were four similar fullbloom stems in each set. Incubation times were:—Set 1, one day;

Set 2, two days; Set 3, four days; Set 4, five days; Set 5, seven days; and Set 6, nine days. At the end of the incubation, Sets 1 and 2 showed no blight symptoms; these later developed normally. Three of the four stems in Set 3 showed mycelial growth and five in a total of 48 blossoms (10.4 per cent) were infected. All stems in Sets 5 and 6 showed infection; Set 5 had 73 per cent of the blossoms infected and Set 6, 80 per cent. From these data it would appear that a period of about three to four days of high humidity is necessary for the infection of blossoms.

These experiments indicate that *Botrytis cinerea* spores are capable of infecting living tissue directly. Darrow and Demaree (8) described a *Botrytis* twig blight of blueberries that killed twig tips and sometimes advanced down the entire new growth of the year. Goheen's (14) study of *Botrytis* twig blight on blueberries described it as a primary pathogen. McKeen (24) in a study of a *Botrytis* cucumber disease stated that infection first becomes established upon dying tissue and then penetrates adjoining healthy tissue. In a study of *Botrytis* infection of lily leaf and bulb infection, MacLean (21) stated that *Botrytis cinerea* is a secondary pathogen following injury and that *Botrytis elliptica* (Berk.) Cooke is a primary parasite on uninjured tissue. In an earlier study of a disease of lily caused by an organism related to or identical with *Botrytis cinerea,* Ward (35) stated that conidia could bring about direct infection of leaves and flower buds, but that mycelium was more effective. Folsom (13) indicated that mycelial cultures were more effective than spores in producing infection by *Botrytis cinerea* in potato tubers. Nelson (29) in a histological study of infection on grapes by *Botrytis cinerea* showed that direct penetration of cuticle by spores was possible by an infection peg and that host cells were separated to a depth of six to ten layers by enzymatic action of the subcuticular mycelium. In another study by Nelson (28), it was shown that direct infection took place in areas containing no lenticels or wounds.

SIMILARITY OF CERTAIN INJURIES AND PHYSIOLOGICAL DISEASES TO BLOSSOM AND TWIG BLIGHT

SEVERAL physiological diseases of the blueberry are similar in appearance to the twig blight disease. During the winter season twigs are subject to sunscald winterkilling. In such cases, the twigs may die back partly as they would when infected by the blight. Severe autumn and spring frosts may injure twigs, blossoms and leaves, especially in low-lying areas. It is possible to distinguish between the blighted plants and those damaged by frost. In the case of autumn frost injury, other kinds of plants in the frost-affected area also show signs of injury, rather than merely the blueberry blighted stems. In the case of spring frost

injury, there are several differences. The blossoms are killed, but show no mycelium which is very much in evidence on blighted blossoms. Frost-injured leaves are all killed, while blight injures only those leaves in contact with infected blossoms. In frost-affected areas other kinds of plants are affected, while only blueberry plants are killed by blight. Twigs suffering from severe salt spray injury are similar in appearance to blighted twigs. Salt injury and winter injury have been demonstrated in the laboratory (17). It is often possible to isolate *Botrytis* from stems injured by frost or salt spray, but the fungus is present there only as a saprophyte.

The effect of lack of pollination can be mistaken for blossom blight, but here also there is a difference. Blighted corollas usually cling to the calyx throughout the summer season, while the corollas of unpollinated flowers fall off. Blighted corollas, after a period of high humidity, plainly show the mycelial growth throughout the summer season. With lower humidity the dried mycelium and conidia remain in evidence on the blossom. The calyx of an unpollinated flower enlarges slightly and becomes deep purple in color, while the calyx of a blighted flower does not change.

PREVALENCE OF THE DISEASE

IT is difficult to estimate the prevalence of the disease in the field because the amount of infection varies with the light intensity, humidity, temperature, varieties of blueberries and soil factors. During the summer of 1951, various methods were used to estimate the amount of infection of blossom and twig blight in apparently representative fields. A random sampling method was attempted in one field where 25 four-square-foot samples were counted in each of three 3-acre plots. A wooden 2- by 2-foot frame was thrown from the center of the plot into various parts of the plot. Wherever the frame landed, the four-foot-square area enclosed was used as a sample area. An attempt was made to have the sample areas equidistant. Thus the sample areas were located more or less at random between the center of the plot and a circle about 100 feet from the center. The stems within each sample area were counted and the per cent of infection was determined.[4] In this manner 100 square feet of each 3-acre plot was examined. The mean per cent of blight for the 3-acre plots was 7.2, 5.5 and 7.9 respectively.

The same three 3-acre plots were sampled in another manner also.

[4] In this paper, unless otherwise stated, per cent of infection or blight indicates the ratio between the number of stems showing blighted blossoms or twigs and the total number of stems in a given area.

Each was divided into 100 subplots. This was done by laying out nine equidistant parallel straight-line strings from end to end and also from side to side, dividing the area into 100 rectangular sections with the same dimensions. In the northeast corner of each subplot a 9-square-foot area was sampled to determine the amount of infection. This method of locating samples may be called the checkerboard method. In those corners where too many weeds or too few blueberry plants were present, no sample was taken. All three plots were sampled in this manner. The mean per cent infection for the three plots was 4.2, 5.3 and 4.2, respectively, listed in the same order as for the random sampling.

In general, environmental conditions in 1952 did not favor blossom blight. In areas where blight had been severe in previous years, very little was found. The checkerboard method of sampling mentioned above was used to examine three 1-acre plots, in which 25 subplots were used. The mean per cent of infection for each of the three plots was 0.0, 0.0 and 1.8, respectively. Although blight was not prevalent in general, many individual varieties or clones were severely blighted.

FACTORS INFLUENCING BLIGHT

Clonal Susceptibility

OF the two principal low-bush species, *Vaccinium angustifolium* and *V. myrtilloides*, there are many forms that occur as naturally spreading clones; their number is unknown. These clones differ markedly in their susceptibility to the disease. Different clones of the same species growing under the same conditions vary from 0 to almost 100 per cent of infection.

While clones differ in susceptibility, the stems within a clone are almost equally susceptible. The range in per cent of infection in each of

TABLE 1

Range and Mean of Blight Per Cent in Ten One-Square-Foot Samples in Each of Ten Different Clones

Clone species	Blight per cent	
	Range	Mean
V. angustifolium	0-0	0.0
" "	0-5	1.9
" "	0-5.5	1.6
" "	0-8	0.8
" "	3-14	9.8
" "	4-15	11.9
" "	35-47	41.9
" "	88-100	96.7
V. myrtilloides	0-0	0.0
" "	0-9	0.9

ten clones in two average fields is shown in Table 1. Each clone was represented by ten 1-square-foot samples picked at random.

The field observations of clonal susceptibility were tested statistically with data obtained from the nursery plantings of blight-susceptible, low-bush clones at Blueberry Hill Research Farm. These data were obtained in two series of plots, one under sawdust mulch and the other under peat mulch. To determine significant differences in the amount of infection among clones, an analysis of variance was applied to each of the two sets of data. Each set of data represented nine clones each replicated four times.[5] A summary of these analyses is presented in Table 2. The first analysis on sawdust plots demonstrates a difference

TABLE 2

Analysis of Variance of Data on Two Sets of Nine Clones Showing Clonal Susceptibility to Blight

Source of variation	d.f.	Mean square	F value
Set 1 (with sawdust mulch)			
Total	35		
Clones	8	9.25	40.4**
Replicates	3	1.30	5.6**
Exp. error	24	.23	
Set 2 (with peat mulch)			
Total	35		
Clones	8	934.94	2.9*
Replicates	3	2389.66	7.6**
Exp. Error	24	314.52	

** Significant at 1 per cent level.
* Significant at 5 per cent level.

TABLE 3

Paired Comparison t Test between Per Cent of Disease in Peat Mulch Plots and Per Cent of Disease in Sawdust Mulch Plots

Clonal type	Mean per cent blight[1]	
	Peat	Sawdust
V. angustifolium (low)	60.50	4.75
" " (half high)	55.75	6.00
" " (low)	47.50	3.75
V. myrtilloides (half high)	41.25	3.25
V. angustifolium (half high)	37.25	5.75
" " (low)	34.75	2.00
" " (low)	34.25	2.25
" " (low)	24.50	2.00
" " (low)	10.75	2.25

$t = 8.22$ at 1% level.
[1] Each number represents mean of 4 replicated plots.

[5] As used here, the number of replicates is the total number of a set of similar plots.

among clones at the one per cent level of significance. The second analysis on peat plots demonstrates a difference among clones at the five per cent level of significance. The range of per cent for each clone is given in Table 3.

Effect of Type of Mulch

The mean per cent infection of clones under peat mulch was 38.5 per cent, while the mean per cent infection of clones under sawdust was 3.5 per cent. This would suggest that the peat mulch, with a greater waterholding capacity, made conditions more favorable for infection.

Using the mean per cent of infection of the four replicates, the nine clones from each treatment were subjected to the paired comparison *t* test. The test was applied to the two mulch treatments to determine any difference in treatments. Table 3 shows that there is a difference between mulch treatments at the one per cent level of significance.

To determine any further association between the two mulch treatments a correlation was applied to the data. Again using the per cent of infection of the four replicates, the nine clones under sawdust mulch were compared with nine clones under peat. A positive correlation coefficient of 0.69 was obtained, this being significant at the five per cent level. This would indicate that the comparative susceptibility of the nine clones was about the same whether under peat or sawdust.

Effect of Time of Application of Fertilizer

It had often been noted that there was more blossom infection in plots receiving an early application of fertilizer than in those receiving a late application. Eglitis, Johnson and Crowley (11) in their study of strains of *Botrytis* pathogenic to the Jersey variety of blueberries stated that plants grown on soils of high fertility appeared to be more susceptible to infection than those grown on soils of a lower nitrogen level. New Maine observations were tested statistically with counts of per cent of blossom infection made on four sets of plots. Each set included plots with no fertilizer and plots where fertilizer treatments were applied on April 24th and May 13th. These were designated as early and late treatments, respectively. On April 24th the flower buds were still in a dormant condition and on May 13th they were in the cluster-bud stage, where the corollas are just beginning to show. The fertilizer was of the 10-10-10 ratio and the rate of application varied somewhat with the set of plots. In all sets of plots there seemed to be a definite trend toward a greater per cent of blossom infection on those plots receiving the early fertilizer. An analysis of variance was made.

The first set of plots consisted of the three treatments replicated 12 times on a mixed clone stand and fertilized at the rate of 350 pounds per acre. In each 4 x 25 foot plot, 20 one-square-foot samples were examined. These samples were taken as a strip in the middle of the plot. Using the mean per cent infection of each plot, a significant difference between treatments at the one per cent level was obtained. Table 4 gives a summary of the analysis. The mean per cent infection for the treatments was 6.9 for the early fertilizer plots, 4.0 for the late fertilizer plots, and 0.82 for the check plots. The treatment L.S.D. at the one per cent level was 2.2 per cent.

TABLE 4

Analysis of Variance of Per Cent of Disease Data After Early, Late, and No Fertilizer Applications, 10-10-10 Ratio at 350 Pounds Per Acre. Twelve Replicated Plots for Each Treatment. Mixed Clone Stand

Source of variation	d.f.	Mean square	F value
Total	35		
Treatments	2	111.15	31.75**
Replicates	11	8.33	3.38*
Exp. error	22	3.50	

* Significant at 5 per cent level.
** Significant at 1 per cent level.

In the second set of plots, with the fertilizer applied at the same rate as indicated above, the plots were two feet square, all on the same clone, and there were six replicates. A significant difference at the one per cent level was found (Table 5). The treatment means were 6.5 per cent infection for the early fertilizer plots, 2.0 per cent for the late fertilizer plots, and 3.0 per cent for the check plots. The treatment L.S.D. at the one per cent level was 3.3 per cent.

TABLE 5

Analysis of Variance of Per Cent of Disease Data after Early, Late, and No Fertilizer Applications, 10-10-10 Ratio at 350 Pounds per Acre. Six Replicated Plots for Each Treatment. All Plots on a Single Clone Stand

Source of variation	d.f.	Mean square	F value
Total	17		
Treatments	2	33.5	10.1**
Replicates	5	6.5	1.9
Exp. error	10	3.3	

** Significant at 1 per cent level.

The other two sets of plots failed to produce any significant differences, but the trend of more blight in the early fertilizer plots remained. The fertilizer was applied at 350 pounds and 175 pounds per acre, respectively, in these two sets of plots.

The data from two sets of 2-foot-square clonal plots that received the 350 pounds per acre rate of fertilizer were combined. This analysis revealed a significant difference between treatments at the five per cent level. The analysis also revealed a significant difference between the two clones at the one per cent level, and a lack of significant difference in interaction of clones and treatments showed that the clones responded similarly to the treatments (Table 6).

TABLE 6

Analysis of Variance of Per Cent of Disease Data in Two Sets of Plots after Early, Late, and No Fertilizer Applications, 10-10-10 Ratio at 350 Pounds per Acre. Six Replicated Plots for Each Treatment in Each Set. Each Set in a Single Clone Stand

Source of variation	d.f.	Mean square	F value
Total	35		
Treatments	2	105.19	3.6*
Replicates	10	53.76	1.8
Clones	1	568.03	19.3**
Interaction	2	10.36	
Exp. error	20	29.44	

* Significant at 5 per cent level.
** Significant at 1 per cent level.

This increased blight might be because an early application, which would be available to the plants[6] after a period of sufficient rain,[7] would increase the succulence and susceptibility of the blossoms. The late application of fertilizer at the cluster-bud stage would not be available to the plant soon enough to have the same effect as the early application.[8]

Effect of Fertilizer Ratio

Forty-four plots, each 4 by 25 feet, received four different fertilizer treatments including a check treatment. The infection data were subjected to a paired comparison *t* test. The fertilizer was applied in the

[6] An increased total nitrogen content of the blueberry plants, after fertilizer was made available to the plants, was found by Prof. Moody Trevett, Dept. of Agronomy, who was in charge of the fertilizer plots.

[7] From April 25th to May 14th precipitation was 2.46 inches.

[8] From May 14th to May 20th (approximate full bloom time) precipitation was 0.13 inches.

fall of 1950 at the rate of 350 pounds per acre in the ratios 1-0-0, 1-1-1 and 1-1-4. The plots were on a mixed clone stand in a randomized complete block design. The degree of fertilizer stimulation was measured by the height of the stems. In the spring of 1951, 300 random stems in each plot were measured and mean heights for treatments were determined. The mean height of stems was 3.57, 4.00, 4.41 and 4.45 inches for check, 1-0-0, 1-1-1 and 1-1-4 plots, respectively.[9]

In July, 1951, 20 one-square-foot samples were examined in a strip in the middle of each plot. Wherever a fertilizer plot fell next to a check plot, the two mean infection percentages were used as paired plots to make up a paired comparison *t* test. It was believed that a paired comparison *t* test was justified in that the same clones would be contained in adjacent plots. In paired comparison of five 1-1-4 fertilizer and five check plots, the fertilizer plots (mean per cent infection of 21.1) were significantly higher at the one per cent level than the check plots (mean per cent infection of 15.4). Where eight plot comparisons were made between the 1-0-0 fertilizer and check plots, no significant difference was found. Although no significant differences from the checks were shown in the 1-0-0 and the 1-1-1 fertilizers, there was a trend for the fertilizer plots to be higher in per cent infection. In eight comparisons, checks averaged 15.46 per cent *vs.* 17.65 per cent for 1-0-0. In seven comparisons, checks averaged 17.3 per cent *vs.* 17.7 per cent for 1-1-1. A summary of some of the statistical data from these fertilizer plots is in Table 7.

ASSAY OF FUNGICIDES

Laboratory Tests

FORTY-ONE fungicides and 12 antibiotics were tested against *Botrytis* spores to obtain basic data on the control of blight. Standard fungicides, experimental fungicides and antibiotics were chosen which might possibly control blight. They were tested at various concentrations. Spores were obtained from nutrient agar plates of *Botrytis* which had been inoculated two to four weeks earlier. The spores were not more than 20 days old. The standard slide germination procedure (7) which was used was modified as suggested by Miller (25), in that the spores were removed from the culture plates and suspended in a 0.2 per cent sucrose, .001 per cent sodium citrate solution which was employed as a spore germination stimulant. One drop of the material being tested was dropped on a hanging-drop microscope slide with a pipette. One drop of a spore suspension containing about 50,000 spores per c.c.

[9] Unpublished data of Moody Trevett, Dept. of Agronomy, Maine Station.

was added to the drop of material under test. This concentration is equal to approximately 35 spores per low-power microscope field. The slide containing the spores and the test chemical was incubated in a moist chamber for 24 hours and then the spore germination per cent was read. The controls which received no fungicides averaged 96 per cent germination. Based on the per cent of germination in the controls, corrections were made for those slides receiving fungicides.

TABLE 7

Paired Comparison t Test between Per Cent of Disease Data in Check Plots and Plots Receiving 1-1-4 Fertilizer Ratio. Each Plot Mean Represents 20 One-Square-Foot Samples on a Mixed Clone Stand

Mean per cent blight	
Check	1-1-4 ratio
29.0	36.9
10.8	16.4
14.7	22.7
11.2	13.5
11.2	15.9

t = 5.38 at 1 per cent level.

The fungicides were tested at various concentrations until tests had been made both above and below the concentration which inhibited spore germination. At each concentration the tests were repeated until two germination percentages were obtained which were less than five per cent apart. The per cent of germination of spores was plotted against the p.p.m. of fungicide on logarithmic probability paper. The E. D. 50 for each fungicide—that amount required to prevent the germination of 50 per cent of the spores—was used as an index of comparison. The term "E. D. 50" is used rather than the older term "L. D. 50" since Finney (12) has pointed out that E. D. (effective dose) is not as restricted a term as is L. D. (lethal dose). The E. D. 50 of fungicides and antibiotics was read from the logarithmic probability graphs. Table 8 lists the E. D. 50 and active ingredients of 37 fungicides. No lines were plotted for two other fungicides, listed at the end of the table which showed no inhibition at 1000 p.p.m. Table 9 lists the E. D. 50 of four antibiotics. Eight antibiotics showed no inhibition of spores at 100 p.p.m. In general, organic mercury compounds, dithiocarbamates, some heterocyclic nitrogen compounds, and some organic sulfur compounds, were more effective than the inorganic metals, inorganic sulfur compounds and antibiotics (Table 8).

The various forms of elemental sulfur were the least effective of

TABLE 8

E. D. 50 as p.p.m. and Active Ingredients of Fungicides Used in Laboratory Assay against Botrytis Cinerea Conidiospores

Fungicide	Active ingredient	E. D. 50 as p.p.m.
Puratized Agric.	Phenyl mercuri triethanol ammonium lactate	0.002
Merculine 100	Phenyl mercury salicylate	0.030
Velsicol 50-CS-46	N-ethyl mercury—1,2,3,6 tetrahydro; 3,6 methano 3,4,5,6,7,7 hexachlorophthalimide	0.088
Dynacide	Phenyl mercury acetate	0.23
Vancide 51	Sodium salt of dimethyl dithiocarbamic acid and sodium salt of 2 mercaptobenzothiazole	0.23
Bioquin	Copper 8 quinolinolate	0.24
Phygon	2.3 dichloro-1, 4-naphthoquinone	0.25
Vancide F995	Manganese salt of dimethyl dithiocarbamate and 2 mercaptobenzothiazole	0.26
Thiram	Tetramethylthiuram disulfide	0.27
Cunilate 2472	Copper 8 quinolinolate	0.31
Cunimene 2528	Zinc salt of an amine complex of copper 8 quinolinolate	0.40
Cunizene 2529	Copper 8 quinolinolate and zinc salt of amine complex of copper 8 quinolinolate	0.47
Dithane D-14 and Ferric Sulfate	Disodium ethylene bis dithiocarbamate and ferric sulfate	0.50
Fermate	Ferric dimethyl dithiocarbamate	0.59
Zerlate	Zinc dimethyl dithiocarbamate	0.61
Tenn. Nu Z	Zinc	1.70
Kolocarbamate	Ferric dimethyl dithiocarbamate and sulfur fused with bentonite	3.70
Orthocide 406	N-trichloromethylthio tetrahydrophthalimide	3.90
Manzate	Manganese ethylene bis dithiocarbamate	4.70
Crag 640	Zinc-copper-chromate complex	5.20
Crag 341	2 heptadecyl glyoxalidine acetate	5.20
Gen. Chem. 1124	Dinitro phenolthiocyanate	6.20
Tenn. Nu M	Manganese	9.00
COCS	Copper oxychloride sulfate	11.8
Crag 658	Copper-zinc-chromate complex	12.7
Velsicol 48-CS-36	1,4 dihydroxy; 5,6,7,8,9,9 hexachloro 5,8 methano 5,8 dihydronaphthalene	13.3
Cop-O-Zinc	Copper and zinc	17.1
Crag 5400	Alpha, alpha-trithiobis (N-dimethylthio formamide)	20.5
Dow 138	Sodium salt of dehydroacetic acid	30.4
Crag 5379	1,2,3 trithio-5, 8-diazacyclonane-4, 9-dithane	50.2
Mathieson 275	Pentachloronitrobenzene	70.5
Dithane Z78	Zinc ethylene bis dithiocarbamate	84.0
Velsicol 48-CS-73	1,4 diketo; 5,6,7,8,9,9 hexachloro; 5,8 methano 1,4,4a,5,8,8a hexahydro-naphthalene	85.0
Dow 137	Dehydroacetic acid	90.5
Gen. Chem. 1189	Oxygenated dimer hexachlorocyclo pentadiene	97.0
Tri-Basic Copper Sulfate	Copper sulfate	130.0
Lime Sulfur	Calcium polysulfide calcium thiosulfate and sulfur	370.0
Mike Sulfur	Sulfur (micronized)	1000+
Kolodust	Sulfur (fused with bentonite)	1000+

TABLE 9

E. D. 50 as p.p.m. of Antibiotics Used in Laboratory Assay against Botrytis Cinerea Conidiospores

Antibiotic	E. D. 50 as p.p.m.
Rimocidin	0.25
Thiolutin	1.50
Streptomycin Sulfate (crude)	11.50
Actidione	22.20
Aureomycin	100+
Terramycin Hydrochloride (crude)	100+
Dihydro Streptomycin Sulfate	100+
Bacitracin	1000+
Chloromycetin	1000+
Potassium Penicillin	1000+
Sodium Penicillin	1000+
Sodium Rimocidin	1000+

the materials tested. There was no inhibition of spore germination with these elemental forms where they were tested at 1000 p.p.m.

Lime sulfur was only slightly more effective than the elemental sulfurs, having an E. D. 50 of 370 p.p.m. Kolocarbamate, a mixture of elemental sulfur and fermate, was less effective than fermate alone. In slide germination fungicidal assay tests with 300 mesh dusting sulfur against *Botrytis* of the *cinerea* type, Wilcoxon and McCallan (37) found that there was no inhibition of spore germination. In another study of germination of spores of the same organism, McCallan and Wilcoxon (23) found that 20 p.p.m. of hydrogen sulfide inhibited the germination of 96 per cent of the spores.

Inorganic compounds containing metals, particularly those containing copper, were not very effective. In 1950, Müller-Stoll (27) reported that spores of *Botrytis cinerea* were markedly insensitive to inorganic compounds containing copper and arsenic.

In the laboratory assay tests reported upon here pentachloronitrobenzene and p-nitrochlorobenzene were also found to be relatively low in toxicity to spores. P-nitrochlorobenzene was available only in the insoluble form and was used as a 25 per cent dust in slide germination tests. Spore response to this material was erratic, but in general there was no effective inhibition. Pentachloronitrobenzene with an E. D. 50 of 70.5 p.p.m. had a comparatively low toxicity. Rich (32) in a study of vapor phase toxicants found that these compounds were completely fungicidal to spores of *Botrytis* sp.

The E. D. 50 values for the antibiotics ranged from 0.25 to 1000+ p.p.m. Rimocidin, Thiolutin, Streptomycin Sulfate, and Actidione with E. D. 50 values of 0.25, 1.50, 11.50 and 22.20 p.p.m., respectively, were the most effective antibiotics. Thanos (33) in a study of the effect of Actidione on spore germination of *Botrytis cinerea* reported that the germination of spores was significantly inhibited at 10 p.p.m., while at 25 p.p.m. a stimulation of spores was noted and at 50 p.p.m. there was no germination. Wallen *et al* (34) reported that growth of *Botrytis cinerea* from spores streaked on agar plates was strongly retarded by 50 p.p.m. of Actidione mixed with the agar.

Greenhouse Tests

The slide germination tests were followed up with greenhouse tests. After the winter rest period of 1951-1952, sods of blueberry clones which had been burned in the spring of 1951 were brought into the greenhouse. These sods were broken up into units containing 20 to 100 stems and were placed in propagation boxes. Half of the stems of a unit were sprayed thoroughly with a fungicide or antibiotic and the re-

maining half were left untreated. Spraying was done by means of a hand atomizer. The normal field concentration for the standard fungicides, the manufacturers' recommendations for the experimental fungicides, and 100 p.p.m. for the antibiotics were used. A total of 21 materials were tested in this manner. At the time of spraying the plants were in full bloom. After the spray had dried on the plants, they were inoculated with a spore suspension by hand atomizer and were placed immediately in a high-humidity chamber. Spores ranging in age from 10 to 30 days were used. They were removed from cultures on nutrient agar plates by flooding with distilled water, and by gently scraping the surface of the medium to detach spores and mycelium. The spore suspension was filtered through cheesecloth to remove the larger pieces of mycelium and was diluted to a concentration of about 100,000 spores per c.c.

The first symptoms of blight were seen seven to eight days after inoculation. The blossoms became brown, and white mycelium was evident on the corollas. It was soon observed that the 10th day was the ideal time for making disease counts because at this time the symptoms were suitably advanced. By the 14th day it was no longer possible to detect the points of original infection because many blossoms had dropped naturally.

The per cent of disease in the treated and check stems was obtained and recorded. The per cent of disease control was ascertained by subtracting the per cent of disease in the treated stems from the per

TABLE 10

Comparison of Laboratory E. D. Values with Disease Control in 1952 Greenhouse Test

Material	E. D. 50 as p.p.m.	Per cent of disease control
Vancide 51	0.23	100
Fermate	0.59	100
Zerlate	0.61	100
Kolocarbamate	3.70	100
Orthocide	3.90	100
Manzate	4.70	100
Vancide F995	0.26	85
Phygon	0.25	78
Dithane Z78	84.0	76
Cop-O-Zinc	17.10	75
Gen. Chem. 1124	6.20	68
Thiram	0.27	67
Crag 658	12.70	60
Thiolutin	1.50	50
Dynacide	0.23	48
Puratized Agric.	0.002	45
Merculine	0.030	45
Crag 640	5.20	28
Crag 5400	20.50	27
Rimocidin	0.25	0
Crag 5379	50.20	0

cent of disease in the check stems, dividing this by the per cent of disease in the check stems, and multiplying the result by 100. The per cent of control with the various tested materials is shown in Table 10.

In greenhouse tests in the spring of 1953, when the blueberry stems had come into full bloom, about 100 blossoms were sprayed thoroughly with each of 37 fungicides and antibiotics. Mike sulfur was the only fungicide tested which had been found to have an E. D. 50 over 1000 p.p.m. Antibiotics with an E. D. 50 of more than 100 p.p.m. were not tested. Two sets of 100 blossoms each were left unsprayed as checks. The spraying procedure was the same as in the previous study. After the spray had dried on the plants, they were inoculated with a spore suspension, which was obtained and used in the same manner as in the 1952 trials. The per cent of disease control was determined on the tenth day of incubation. The best means of determining the per cent

TABLE 11

Comparison of Laboratory E. D. 50 Values with Disease Control in 1953 Greenhouse Test

Material	E. D. 50 as p.p.m.	Per cent of disease control
Fermate	0.59	94
Vancide F995	0.26	91
Orthocide	3.90	90
Crag 5400	20.50	90
Gen. Chem. 1124	6.20	88
Phygon	0.25	86
Zerlate	0.61	76
Crag 5379	50.20	73
Manzate	4.70	69
Dithane D-14 and Ferric Sulfate	0.50	60
Velsicol 48-CS-36	13.3	60
Thiram	0.27	56
COCS	11.80	55
Tenn. Nu Z	1.70	50
Vancide 51	0.23	47
Kolocarbamate	3.70	44
Puratized Agric.	0.002	42
Crag 658	12.7	42
Crag 640	5.20	36
Cunizene 2529	0.47	29
Merculine	0.03	28
Tenn. Nu M	9.00	28
Cop-O-Zinc	17.10	26
Lime Sulfur	370.0	26
Dow 138	30.4	22
Crag 341	5.20	22
Dithane Z78	84.0	19
Cunilate 2472	0.31	19
Mike Sulfur	1000+	17
Dynacide	0.23	15
Cunimene 2528	0.40	15
Tri-Basic Copper	130.0	13
Velsicol 48-CS-73	85.0	12
Bioquin	0.24	11
Actidione	22.20	9
Gen. Chem. 1189	97.00	8
Rimocidin	0.25	6
Thiolutin	1.50	5
Terramycin	100+	3
Mathieson 275	70.5	0
Dow 137	90.5	0

of disease was found to be counting the amount of calyx infection, inasmuch as the corollas tended to fall off.

The percentage of control obtained is shown in Table 11.

Some Comparisons

To compare the results of the 1952 and 1953 greenhouse assays, a correlation was made on the per cent of disease control obtained by the 21 materials in 1952 and the per cent of disease control for the same 21 materials in 1953. A positive correlation of 0.41 indicated about the five per cent level of significance. Although the 1952 and 1953 greenhouse tests were similar in result, they were not exactly comparable with the laboratory assay tests. Perhaps one reason for this was the different weathering properties of the materials when subjected to the mist in the humidifier chamber. However, the materials which were high in laboratory E. D. 50 values were low in greenhouse per cent of disease control, and the materials which were relatively low in E. D. 50 values gave the best disease control with the exception of the organic mercuries.

Campbell (6) in 1949 reported that dusts of sulfur, Fermate, Zerlate, Phygon and copper lime gave no control of grey mold disease of beans caused by *Botrytis cinerea*. Calavan *et al* (5) reported that of eight fungicides, including Crag 640, Crag 658, Dithane D-14 and copper sprays, used to control blossom blight of lemons caused by *Botrytis cinerea,* Dithane D-14 gave the best control (5 per cent infection as compared to 22 per cent infection in the check) and that copper-bearing sprays (10 per cent infection) gave limited protection. In a study of *Botrytis cinerea* infection of strawberries, Powell (31) found that Fermate, Orthocide and Phygon gave better control than sulfur and Bordeaux mixture. Gladiolus *Botrytis* leaf-spot disease studies by Holloman and Young (19) showed that of 16 chemicals tested in petri plate spore germination tests five showed sufficient promise to warrant field testing. In field tests Fermate, Dithane D-14, Phygon and Puratized gave disease indices of 13.86, 18.61, 29.15 and 33.85, respectively, as compared to a check index of 44.62. Crag 341 gave an index of 54.39, as compared to 62.07 in the check. These results were significant at the one per cent level. In 1951 Palmiter (30) reported that Fermate and Orthocide gave the best protection against a blossom end rot of apples caused by *Botrytis cinerea*. Sulfur provided no protection, and Puratized, Dynacide, Phygon and Crag 341 gave some protection. Wilhelm (38) in a study of *Botrytis* grape decay control indicated that Bordeaux mixture gave no control on injured grapes but reduced disease on uninjured grapes. Horn (20) in experiments on control of *Botrytis cinerea* strawberry disease stated that of 41 fungicides tested, three gave good control.

These three materials were a salt of dihydroacetic acid (similar to Dow 137 and Dow 138 reported on here), Crag 5400 and Orthocide. Anderson and Gottlieb (1) reported that Actidione showed some effect when used as a systemic fungicide. Lettuce plants grown in water culture containing ten micrograms of Actidione showed 40 per cent disease when inoculated with *Botrytis cinerea,* as compared to 100 per cent infection in the control.

Some of the fungicides used in the greenhouse injured the blueberry leaves. Puratized, Merculine, Dynacide and Actidione caused severe leaf injury. In early July of 1951 when the fruit had set, 14 fungicides applied as concentrate sprays were tested in the field for phytotoxic effects. The materials were applied to rod square plots replicated five times. Puratized caused severe defoliation, Tri-Basic Copper Sulfate caused some defoliation and Cop-O-Zinc and Crag 341 caused some leaf injury. D. W. Creelman of the Dominion Experimental Station at Kentville, Nova Scotia, tested 11 materials on low-bush blueberries (*V. angustifolium*) for phytotoxic effects.[10] Two sprays were applied, one at bloom and one when fruit had set. Bordeaux mixture treatments caused no injury. Basicop spray gave scorched appearance to the foliage. Of the organic fungicides not containing mercury that were tested (Phygon, Zerlate, Fermate and Crag 341), only Crag 341 was injurious. It caused almost immediate blossom injury. Puratized, Tag and phenyl mercury chloride produced immediate injury to leaves causing them to turn brown and drop off. Succulent shoots were killed and new growth suppressed.

Field Trials

A field test of fungicides for control of the blight was attempted in 1952. Seven materials previously tested in the laboratory and greenhouse were applied respectively to each of seven approximately one-acre plots. Three applications were made, the first on May 14th at the prebloom stage, the second on June 4th during full bloom, and the third on June 23rd when most of the fruit had set. Since very little disease was present, it was possible only to obtain data which showed trends of disease control. Each plot was carefully searched until the five most infected clones were found. Each clone was marked and the per cent infection in 10 square feet of the clone determined. In most cases only three or four clones showed infection, while in other cases only one infected clone could be found. Table 12 shows the materials tested and mean per cent blight of each treatment including a nontreated check.

So far as could be determined, Fermate, Dithane D-14 (with ferric

[10] Unpublished progress report.

sulfate), and Phygon gave almost 100 per cent control. Parzate liquid (a form of Dithane D-14), with zinc sulfate, gave 86 per cent control, Zerlate gave 69 per cent control, Manzate gave 33 per cent control and Crag 658 dust gave no control.

Most of these materials were tested again in 1953 and similar disease control was obtained. A new material, Vancide ZW, was included in the 1953 trials and gave control of blight almost equal to that obtained with Fermate.

The disease control obtained in the field with fungicides seemed to follow somewhat the same trend as is indicated by laboratory and greenhouse testing of the same materials.

TABLE 12

Mean Per Cent of Blight and Per Cent of Disease Control in Five Ten-Square-Foot Samples in Each of Eight Approximately One-Acre Plots

Material	Amount per acre	Mean per cent blight	Per cent of disease control
Crag 658, 6% dust	15 lbs.	9.84	0
Manzate, 5.2% dust	15 lbs.	4.66	33
Zerlate, 2 lbs. in 12 gals.[1]	12 gals.	2.30	69
Parzate liquid, 2 qts. and Zinc Sulfate, 0.75 lb. in 12 gals.[1]	12 gals.	1.00	86
Fermate, 7.6% dust	15 lbs.	0.18	97
Phygon, 0.5 lb. in 12 gals.[1]	12 gals.	0.12	98
Dithane D-14, 1.5 qts. and Ferric Sulfate, 1 lb. in 12 gals.[1]	12 gals.	0.08	99
Check		6.96	

[1] Applied as concentrate mist spray at 8x concentration.

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